Synopsis

Karyomorphology and induced mutagenesis in *Lens culinaris* Medik. through Sodium azide for chromosomal aberrations and production of bioactive peptides

Submitted by:

Prerna Kumari

(M. Sc.)
(Qualified R. U. Ph.D. Entrance Exam Roll No.: 03025)
University Department of Botany
Ranchi University, Ranchi

Under the Supervision and Guidance of

Dr. Sameer Gunjan Lakra
Assistant Professor
University Department of Botany
Ranchi University, Ranchi

2025

STATE OF THE ART

The genus *Lens culinaris* Medik. belongs to the legume family Fabaceae and comprises self-pollinated, diploid species having chromosome number 2n=14[1,2-3]**.** It is an annual**,** pale green herbaceous plant generally 20-45 cm in height[4]. The plant generally shows an indeterminate flowering and branching type of growth pattern, a slender, soft-haired angular stem, pinnate leaves, and produces oblong flattened pods having one or two biconvex rounded seeds per pod[4]. Its vernacular name is 'Lentil', - in English because of its lens-shaped seeds; 'Masoor and Masur dal' in Hindi, Urdu, and Marathi; 'Masuri' in Bengali; 'Chanagi' in Kannada; 'Misur paruppu' in Tamil; 'Masur pappu' in Telugu, etc[5]. Lentil is one of the ancient domesticated, nutritious leguminous crops**,** generally grown as a rabi crop[6]. Globally lentils rank sixth in major pulse production after dry bean, fava bean, pea, chickpea, and cowpea (FAO,2023)[7]. The plant is indigenous to Western Asia but is now worldwide cultivated in over 52 countries[8]. India is the second-highest leading producer of lentils after Canada, followed by Turkey, Australia, Nepal, USA, Bangladesh, Syria, Iran, and Ethiopia (FAOSTAT, FAO Statistics division 2021[9], 2018[4])**.** In India, lentil is primarily grown as a rabi crop in some states of the eastern and northern region such as Uttar Pradesh, Bihar, Haryana, Madhya Pradesh, West Bengal, Rajasthan, Punjab, Assam, Maharashtra, etc[4]. In the progressive world, nutraceuticals and functional foods have gained attention exponentially, as they are the novel approach in the prevention and cure of chronic diseases[10-11]. Lentil is regarded as a natural "functional food" due to its attractive nutritional composition, ethno-pharmacological profile and bioactive secondary metabolites[11]. Its nutritional composition includes approximately 60% carbohydrate and 25% protein as major dietary components, rich in dietary fibers, minerals (such as iron, folate, magnesium, and zinc, etc.), essential amino acids (such as lysine and arginine), and globulin and albumin are the major storage proteins[12]. Lentils epitomize the source of plant-based protein to the vegetarian population as an alternative to animal-based protein[12-13]. Ethnopharmacological constituents of lentils include polyphenols (such as phenolic acid and flavonoids), saponins, phytosterols, and other bioactive compounds are trypsin/protease inhibitors, lectins, defensins, dietary fibers, etc[14-15]. Therefore, the consumption of lentils and lentil-based products lowers the incidence of chronic diseases and has potential health benefits such as anti-diabetic, anti-oxidant, anti-obesity, cardioprotective and anti-cancer activity[1,16,17-18]. Legumes are

considered as one of the primary sources of amino acids for humans due to their higher protein content i,e., 2 to 3 times that of wheat and rice[19]. Globulins such as vicilin and legumin are storage proteins in lentils which contribute about 72% and 11% of total protein content respectively[19]. Lentil protein serves not only as a major source of dietary amino acids but also as a source of bioactive peptides[20]. Bioactive peptides are organic compounds composed of a stretch of amino acids joined by peptide bonds[21]. These peptides are derived from natural sources (plants, fungi, microbes, animals etc.) and are released when the proteins are broken down by enzymatic hydrolysis or during the processing of foods such as fermentation, cooking, and ripening[21]. Recently, considerable interest has been devoted to the production and applications of bioactive peptides, as they become novel drug candidates in the current drug development pipeline due to their inherent property of causing physiochemical changes in normal cellular processes[21,22,23-24]. These properties of bioactive peptides result from their unique sequence, composition, and molecular weight of amino acid[8]. Bioactive peptides regulate numerous essential bodily functions such as antihypertensive activity as they act as a competitive inhibitor of angiotensin-converting enzymes (ACEs); antimicrobial; anti-thrombotic; immune-modulatory; lowering cholesterol by inhibiting cholesterol biosynthesis pathway; prevent type 2 diabetes by controlling differentiation of adipocyte and lipase activity and by inhibiting enzyme such as dipeptidyl peptidase IV (DPP-IV), alpha-amylase and alpha-glucosidase, and anti-cancerous activity by exhibiting necrosis, apoptosis, inhibiting angiogenesis, and promotes pore-formation etc,[20-23]. The nutritional composition and nutritive values of lentils between different varieties are influenced by their genetic constitution and combined effect of agricultural practices, and their environmental conditions[26]. Thus, cytological knowledge is the primary and valuable technique for planning and designing effective breeding strategies for creating genetic diversity among crops and determining the closeness between different varieties to select the parental lines for crop improvement[27]. The world lentil production report in 2012 by FAO, 2014 was 4,557,972 metric tons from an area harvested of 4,206,024 hectares, and India alone contributes 20.84 percent of total world production, but grain yield is only 0.5938 tons per hectare, which is very low in comparison to the world scenario, i.e., 1.0837 tons per hectare[4]. This indicates that despite tremendous agricultural practices and breeding approaches, low yields continue to be a significant challenge[28]. To overcome these challenges, desirable genetic variation is required to create genetic diversity[28-29]. Thus, induced mutation through chemical mutagen is one of the coherent tools for broadening

genetic variation and breaking the genetic bottleneck of lentil genotypes[30-31]. Overall, a combination of unique nutritional profile, health benefits, and agricultural and economic advantages make *Lens culinaris* Medik. a focal point for researchers[1]. So, in the current study, we have approached to perform comparative karyomorphological studies of different species or varieties of *Lens culinaris Medik.* that provide insight about genetic variations, variations in evolutionary patterns, and phylogenetic relationships to identify the primitive and advanced varieties[32-33]. Through, a further breeding approach, the primitive varieties may be further used as a precursor plant to develop crops with desired traits. Crops with desired traits can also be developed through a conventional breeding approach but it is time-consuming and laborious process. So, we generally get reliable on chemical mutagens for crop improvement. Thus, the selected species or varieties obtained through karyomorphological studies will be treated with chemical mutagen i.e. sodium azide, which is a potent chemical that can improve the quality and yield of crops[34], to observe the chromosomal abnormalities, its resistance as well as the sensitivity of the plant towards it. In addition to that the synthesis of bioactive peptides and the effect of chemical mutagen with different doses on the proteins of the selected varieties will also be analyzed.

PROBLEM TO BE INVESTIGATED

Karyotype analysis along with different parameters will be performed on varieties of *Lens culinaris* Medik. to construct an evolutionary pattern. The effect of sodium azide will be analyzed on the few varieties of *Lens culinaris* Medik. at the chromosomal level and active biopeptide synthesis.

OBJECTIVE OF INVESTIGATION AND MOTIVATION

OBJECTIVES OF THE PRESENT WORK ARE:

1. To study the detailed karyomorphological aspects of collected seed varieties of *Lens culinaris* Medik.
2. To evaluate the effect of chemical mutagen on the selected varieties of *Lens culinaris* Medik.

3. To evaluate the anticancer properties of bioactive peptides derived from control and treated plants of *Lens culinaris* Medik.

MOTIVATION:

The genus *Lens culinaris* Medik. comprises diploid self-pollinated species with chromosome number 14[2,3]. It is a nutritionally dense and protein-rich legume crop, generally grown in winter[6]. Lentils are regarded as a "functional food" among pulses due to their nutraceutical and therapeutic values[14]. Its nutritional make-up includes the highest potential source of proteins, fibers, antioxidants, bioactive peptides, polyphenols, resistant starches, prebiotic carbohydrates, phytochemicals, vitamins (thiamine, riboflavin, niacin, pantothenic acid, folate, pyridoxine, alpha, beta, gamma tocopherols, and phylloquinone), and minerals (zinc, copper, manganese, molybdenum, etc.)., etc[12-14]. Total protein content in lentils ranges from 28.7 to 31.5 percent of total weight[12]. Consumption of lentils helps to regulate blood cholesterol and diabetes due to the valuable amount of dietary fibers and complex carbohydrates such as saponins and lectins, which have anti-cancerous activity[1,15]. Lentils contain a lower content of fatty acids; they are cholesterol-free, gluten-free, and rich in essential and non-essential amino acids; they are a potential source of probiotics; prebiotic carbohydrate helps to improve the gut microbial environment; and iron prevents anemia[12]. Other phytochemicals predominant in lentils as phytosterols are beta-sitosterol, campesterol, stigmasterol, and saponins are associated with the reduction in the incidence of several degenerative diseases[20,25]. Lentils are also rich in peptides such as protease inhibitor proteins such as bowman-birk type, trypsin inhibitor, angiotensin-1-converting enzyme inhibitor, defensin, etc., that have a positive effect on inflammatory diseases and in the treatment of cancer diseases[17,21,25]. Bioactive peptides are gaining attention in drug development because of their appealing properties like smaller in size and high oral bioavailability, high specificity and potency, fewer side-effects and immunogenicity than larger proteins, easy and less complex production process, enhanced tissue penetration, low toxicity and easily modified[21]. The above nutraceutical composition and health benefits increase the scientific interest of lentils among researchers[1].

HYPOTHESIS

Comparative karyomorphological studies of varieties of *Lens culinaris* Medik**.** are performed to determine the similarities and differences in the karyotype and to observe the basic sets of chromosomes in each variety[35-36]. The basic purpose of this study is to evaluate the intervarietal relationship as well as to frame the evolutionary pattern between varieties of Lentils, to select primitive or advanced varieties for the development of superior plant [32,37].

Treatment of selected varieties with chemical mutagen will reveal the dose at which sodium azide supports crop improvement and also determines whether the plant is either sensitive or resistant to sodium azide. The effect of sodium azide on the proteins leads to the synthesis of either functional or altered bioactive peptides which may show anti-cancerous properties.

RESEARCH DESIGN AND METHODOLOGY

- **COLLECTION OF SEED MATERIAL:**

The seeds of *Lens culinaris* Medik. will be obtained from Agricultural University and seed center.

- **CYTOLOGICAL STUDIES:**

The healthy seeds of lentils will be used for cytological studies. The seeds will be soaked and allowed to germinate in sterilized tap water. Root tips will be excised at appropriate time when it attains a length of 1-2 cm. For cytological studies, excised root tips were pretreated with paradichlorobenzene. Then pretreated root tips will be thoroughly washed with distilled water and fixed with carnoy's fluid and preserved in Ethanol. Preserved tips will be stained in 2 percent Acetocarmine and temporary slides were prepared by smear and squash technique[31].

For karyomorphological studies, the length and size of the chromosome will be studied at the metaphase stage with the help of a stage micrometer and ocular scale and mitotic slides will be observed under microscope. Through the preparation of ideograms, histograms, and graphs we can calculate absolute length, relative length, angular values, log values, total chromatin index, symmetry index, anaphase index, and telophase index[38].

- **TREATMENT WITH SODIUM AZIDE, A CHEMICAL MUTAGEN:**

For the preparation of stock solution, 0.065 gm of Sodium azide is dissolved in 100 ml of distilled water to prepare a stock solution of strength of 0.01M. This stock is diluted to different degrees to prepare 5 different concentrations of sodium azide to analyze its mutagenic effects on M1 and M2 generation on a few species or varieties of *Lens culinaris* Medik.

Excised root tips of lentils will be pretreated, fixed, and preserved for further studies of chromosomal aberration.

- **BIOACTIVE PEPTIDE SYNTHESIS**

  Lentil protein extract preparation

  Control and treated seeds will be grounded and then defatted for protein extract preparation[39-40].

  Hydrolysis of lentil proteins

  Biopeptides will be generated through specific proteases such as alcalase, flavourzyme, bromelain, pepsin, and trypsin etc[39].

  Isolation and Purification of peptides

  Extraction and purification of peptides will be done through several processes such as ultrafiltration and dialysis[39,41].

  Characterization of peptides

  This work will be done through LC-MS.

  Anti-cancer activity

  Anticancer activity will be done either through Enzyme Inhibition Assay, Reactive Oxygen Species Assay or Bioinformatics Approaches.

TOOLS TO BE USED IN DATA COLLECTION

Beaker, Glass rod, spirit lamp, Slides, Coverslip, Compound microscope, Stage micrometer, Ocular, Scale, Digital balance, Measuring cylinder, Sodium-azide, Carmine, centrifuge, pH meter, Book, Journals and computer, etc.

SIGNIFICANCE OF THE STUDY

- Karyomorphological studies are performed for the identification and characterization of different varieties of *Lens culinaris* Medik**.** which is the prerequisite information for breeding in crop improvement programmes[42].
- Comparative karyomorphological studies provide information about origin, ploidy, phylogenetic, and evolutionary relationships among varieties which are used by the breeders to manipulate the genome to overcome the problem[43.]
- Plant mutagenesis plays a significant role in the enhancement of genetic variation through exposure to chemical mutagen. This may cause desirable chromosomal manipulation without undesirable genetic alterations[44].
- Exposure to chemical mutagens may lead to the generation of functional biopeptides having an anti-cancerous activity which is further exploited by drug and pharmaceutical industries.

IT'S RELEVANCE TO THE PRESENT DAY PROBLEM AND NEED TO THE SOCIETY

Through comparative karyomorphological studies between varieties, we can interpret genetic variations and the chromosomal basis of desirable traits[45]. Identifying the precursor plant with compatible karyotypes is the primary requirement for successful hybridization and the production of new varieties with superior traits. This also helps the breeders to identify and eliminate the undesirable varieties. Karyomorphological studies provide a clear idea about phylogenetic and evolutionary relationships by understanding chromosomal similarities and differences within different varieties to select desired genetic material for the production of transgenic crops[46]. Sodium azide is a potent chemical mutagen, as it produces mutagenic metabolite which enters the nucleus, interacts with DNA, and causes point mutation, which can be helpful in the creation of genetic diversity and the development of desirable traits[47-48]. Globally, the lifestyle-related chronic diseases such as cancer is one of the major causes of morbidity and mortality in the recent developmental era. At present, chemotherapy, radiotherapy, immunotherapy, and surgery are some of the most effective approaches for the treatment of cancer. However, these therapeutic approaches are associated with severe side effects. So, there is a need for safer alternatives that should kill the cancer cells without hampering healthy neighboring cells.

Therefore, this study will be focused on the synthesis of biopeptides and investigating the effect of chemical mutagens on the protein isolates and its role as an anti-cancer agent.

CONTRIBUTION TO THE EXISTING KNOWLEDGE

- Comparative karyomorphological studies between selected varieties of *Lens culinaris* Medik**.** provide insight into genetic variations, variations in evolutionary patterns, and phylogenetic relationships to identify primitive and advanced varieties for breeding programs in crop improvement techniques[49-50].
- Through mutational breeding, with the help of chemical mutagen sodium azide, plant breeders may develop new varieties of crops with improved traits such as tolerance to biotic and abiotic stress, greater yield, adaptability to unfavorable conditions, etc.
- The dose of sodium azide might be optimized to check the extent of sensitivity and resistivity in *Lens culinaris* Medik**.** through the monitoring of chromosomal aberrations in two subsequent generations.
- Sodium azide is a potent mutagen that causes point mutation that may lead to alteration in the protein due to changes in the sequence of amino acids. As, a result it may generate functional or non-functional peptides with or without anticancer potential. It will lead to the discovery of therapeutic peptides with health benefits.

FUTURE PROSPECTS

1. To determine the genes responsible for desired traits such as better yield and to withstand unfavorable conditions like abiotic and biotic stress, changing environmental conditions in lentils.
2. Development of productive plant varieties through genomic selection technique.
3. Development of biofortified lentil varieties through genome editing technology.
4. To determine specific peptides responsible for anti-cancerous activity and its mode of action.

REFERENCES

1. Ganesan, K., & Xu, B. (2017). Polyphenol-rich lentils and their health promoting effects. *International Journal of Molecular Sciences*,18,2390.
2. Jha, B. T., Bhowmick, K. B., & Roy, P. (2021). Analysis of CMA-DAPI bands and preparation of fluorescent karyotypes in thirty Indian cultivars of *Lens culinaris*. *Caryologia,*74(2):65-77.
3. Prashanth, B. C., Geetha, M. K., Wilson, B., & Banu, S. (2024). Traditional uses, bioactive composition and pharmacological activities of *Lens culinaris*. *South African Journal of Botany*, Volume 168, Pages 542-561.
4. Kumar, J., Kumar, S., & Siddique, HM. K. (2024). The Lentil genome: genetics, genomics and breeding. DOI: https:\\doi.org\10.1016\B978-0-443-19409-2.00015-6.
5. Nair, P. K. (2019). Utilizing crop wild relatives to combat global warming. *Advances in Agronomy.*
6. Singh, M. K., & Singh, K. A. (2014). Lentil in India: An Overview.
7. FAO,2023. FAOSTATE Database. Food and Agriculture Organization of the United Nations, Rome. Available from: https:\\faostat.fao.org.
8. Sun, Li., Liu, J., He, Z., & Du, R. (2024). Plant-derived as alternative to animal-derived bioactive peptides: A review of the preparation, bioactivities, structure-activity relationships, and applications in chronic diseases. *Nutrients*, 16, 3277.
9. Choudhary, M., & Sharma, A. (2022). A review on breeding, global production and utilization of lentil. *The Pharma Innovation Journal*, SP-11(5):1303-1310.
10. Aslani, Z., Alipour, B., Mirmiran, P., & Bahadoran, Z. (2014). Lentils *(Lens culinaris L.)* functional properties in prevention and treatment of non-communicable chronic diseases: a review. *International Journal of Nutritional and Food Sciences*,4(2-1):15-20.
11. Irakli, M., Kargiotidou, A., Tigka, E., Beslemes, D., Fournomiti, M., Pankou, C., Stavroula, K., Tsivelika, N., & Vlachostergios, N. D. (2021). Genotypic and environmental effect on the concentration of phytochemical contents of lentil (*Lens culinaris L.*) *Agronomy,*11,1154.
12. Mustafa, M. A. Abouelenein, D., Acquaticci, L., Alessandroni, L., Angeloni, S., Borsetta, G., Caprioli, G., Nzekoue, K. F., Sagratini, G., & Vittori, S. (2022). Polyphenols, saponins and phytosterols in lentils and their health benefits: an overview. *Pharmaceuticals,*15,1225.
13. Liberal, A., Almeida, D., Fernandes, A., Pereira, C., Ferreira, CFR. I., Vivar-Quintana, M. A., & Barros, L. (2023). Nutritional, chemical, and antioxidant screening of selected varieties of lentils *(Lens culinaris spp.)* from organic and conventional agriculture. *Journal of The Food and Agriculture*.
14. Turi, Di. A., Antonacci, M., Dibenedetto, R. J., Maqoud, F., Leonetti, F., Centoducati, G., Colonna, N., & Tricarico, D. (2023). Molecular composition and biological activity of a novel acetonitrile-water extract of *Lens culinaris Medik* in murine native cells and cell lines exposed to different chemotherapeutics using mass spectrometry. *Cells*,12,575.

15. Faris, Al-I. M., Mohammad, G. M., & Soliman, S. (2020). Lentils (*Lens culinaris L.*): a candidate chemopreventive and antitumor functional food. *Functional Food in Cancer Prevention and Therapy,* 99-120.
16. Dewan, F. Md., Shams, Nur-Us. S., & Haque, A. M. (2024). A review of the health benefits of processed lentils (*Lens culinaris L.*). *Legume Sciences* 6: e232.
17. Alexander, R., Khaja, A., Debiec, N., Fazioli, A., Torrance, M., & Razzaque, S. M. (2024). Health-promoting benefits of lentils: Anti-inflammatory and anti-microbial effects. *Current Reseach in Physiology*. 7,100124.
18. Gharachorloo, M., Tarzi, G.B., Baharinia, M., & Hemaci, H. A. (2012). Antioxidant activity and phenolic content of germinated lentil *(Lens culinaris*). *Journal of Medicine Research* Vol. 6 (30), pp. 4562-4566.
19. Kumar, S., Divya., Thakur, S., Verma, M., Hajam, A.Y., Kumar, R., Dhull, B. S., Rose, K. P., Muzaffar, N., & Gautam, D. (2024). Nutraceutical and pharmacological benefits of some leguminous plants of North-Western Himalaya. *Legume Science* 6: e236.
20. Alzaydi, A., Barbhuiya I.R., Routray, W., Elsayed, A., & Singh, A. (2023). Bioactive peptides: synthesis, application, and associated challenges. *Food Bioengineering*,2:273-290.
21. Purohit, K., Reddy, N., & Sunna, A. (2024). Exploring the potential of bioactive peptides: from natural sources to therapeutics. *International Journals of Molecular Sciences* 25, 1391.
22. Li, R., Hettiarachchy, N. & Mahadevan, M. (2014). Rice bran derived pentapeptide induced apoptosis in human breast cancer cell models (MCF-7 and MDA-MB 231). *International journal of Biomedical Research*, 5(10), pp. 599-605.
23. Hilchie, A.L., Hoskin, D.W. & Power Coombs, M.R. (2019). Anticancer activities of natural and synthetic peptides. *Antimicrobial peptides*, pp.131-147.
24. Kurrikoff, K., Aphkhazava, D. & Langel, Ü. (2019). The future of peptides in cancer treatment. *Current Opinion in Pharmacology*, 47, pp.27-32.
25. Fuentes, R. L., Richard, C., & Chen, L. (2021). Sequential *alcalase* and *flavourzyme* treatment for preparation of alpha-amylase, alpha-glucosidase, and dipeptidyl peptidase (DPP)-IV inhibitory peptides from oat protein. *Journal of Functional Foods*, 87,104829.
26. Liberal A., Fernandes, A., Ferreira, C.F.R. I., Vivar-Quintana, M. A. (2024). Effect of different physical pre-treatments on physicochemical and techno-functional properties, and on the antinutritional factors of lentils (*Lens culinaris spp*.). *Food Chemistry* 450,139293.
27. Pal. T., Ghosh, S., Mondal, A., & De, k. k (2016). Evaluation of genetic diversity in some promising varieties of lentils using karyological characters and protein profiling. *Journal of Genetic Engineering and Biotechnology*14, 39-48.
28. Shahwar, D., Ansari, M.Y.K., Khatoon, B., & Park, Y. (2024). Phenotypic characterization of ethyl methanesulfonate (EMS) induced bigger pod (bp) with multiple seed mutant in lentil (*Lens culinaris Medik*.) *Biocatalysis and Agricultural Biotechnology*, 59, 103259.

29. Laskar, A.R., Laskar, A. A., Raina, A., Khan, S., & Younus, H. (2018). Induced mutation analysis with biochemical and molecular characterization of high yielding lentil mutant lines. *International Journal of Biological Macromolecules*, volume 109, pages 167-179.
30. Taziun, T., Laskar, A. R. Amin, R., Khan, S., & Parveen, K. (2018). Effects of dosage and durations of different mutagenic treatment in lentil (*Lens culinaris Medik.*) cultivars Pant L 406 and DPL 62. *Legume Research*, 41(4):500-509.
31. Joshi, S., & Kumar, K. (2017). Comparative cytological studies of two varieties of *Lens culinaris.* RUJOST, ISSN: 2319-4227, Vol.4, No.2(ii), July, 2017, pp.13-16.
32. Parvatham, G. & Rangasamy, S.R. (2004). Karyomorphological and phylogenetic studies in different species of sorghum L. Moench. *,Cytologia,* 69(3): 301-305
33. Lakra, S.G., & Kumar, K. (2020). Karyomorphology of three succulent species *Gasteria batesiana, Haworthia limifolia var longiana* and *Aloe jucunda* (family Asphodelaceae). *The Journal of the Indian Botanical Society*, volume 99, Issue 3&4, Pages 167-172.
34. Khan, S., Qurainy, F.A., Anwar, F. (2009). Sodium azide: - a chemical mutagen for enhancement of agronomic traits of crop plants. *Environ. We Int. J. Sci. Tech*. 4 ,1- 21 (its relevance to the society).
35. Sun, W., Wang, H., Wu, R., Sun, H., & Li, Z. (2020). Karyomorphology of three endemic plants (Brassicaceae: Euclidieae and Arabideae) from the Qinghai-Tibet Plateau and its significance. *Plant Diversity* 42 135-141.
36. Lakra, S.G., & Kumar, K. (2015). Cytotaxonomy of *Trigonella foenum-graecum L.* collected from Jharkhand and Uttar Pradesh*. *International Journal of Bioassays*, Volume4, Issue 01, Pages 3670-3672.
37. Ismat, S., & Kumar, K. (2017). Comparative karyological studies of two varieties of *Zea mays* L. collected. Ranchi University Journal of Science and Technology*RUJOST*, ISSN: 2319-4227, Vol.4, No.2(ii), July, 2017, pp.35-38.
38. Mahato, U., & Kumar, K. (2017). Cytological studies on three species of *Aloe* collected from Ranchi, Jharkhand. RUJOST, ISSN: 2319-4227, Vol.4, No.2(ii), pp.1-6.
39. Xu, X., Qiao, Y., Shi, B., Dia, V. (2021). Alcalase and bromelain hydrolysis affected physicochemical and functional properties and biological activities of legume proteins. *Food Structure*, 27, p.100178.
40. Naiker, T., Gerrano, A., Mellem, J. (2019). Physicochemical properties of flour produced from different cowpea (Vigna unguiculata) cultivars of Southern African origin. *Journal of Food Science and Technology*, 56(3), pp.1541-1550.
41. Gu, C., Song, X., Zhao, L., & Quin, G. (2014). Purification and characterization of Bowman-Birk Trypsin Inhibitor from soyabean. *Journal of Food and Nutrition Research*, Vol. 2, No. 9, 546-550.
42. Kyani, S., Akrami, H., Mostafaei, A., Akbari, S., & Salehi, Z. (2021). Inhibitory effect of Bowman-Birk protease inhibitor on autophagy in MDAMB231 breast cancer cell line. *J Can Res Ther*, 17: 504-9.
43. Warasy, A.A. (2020). Comparative karyomorphological analysis of two varieties of Brassica compestris from Bangladesh. *J. Biol. Sci.* 9 (1&2) :69-77
44. Naaz, N., Choudhary, S., Sharma, N., Hasan, N., Alshaye, N.A. & El- Monein, D. A. (2022). Frequency and spectrum of M2 generation and genetic variability in cyto-

agronomic characteristics of fenugreek induced by caffeine and sodium azide. *Front. Plant sci.* 13.1030772. doi:10.3389/fpls.2022.1030772.

45. Astuti, G., Marzio, F. R & Peruzzi, L. (2017). Traditional karyomorphological studies: can they still provide a solid basis in plant systematics? **F1. Medit*. 27:91-98 doi:10.7320/F1Medit27.091.
46. Ravindran, A., & Das, B. N. (2022). Karyomorphological studies in two species of genus *Strychnos*. L. significant in ethnopharmacological aspects. *Indian J. Genet. Plant Breed.,* 82(1): 121-124.
47. Alemayehu, G.F., Forsido, S.F., Tola, Y.B & Amare, E. (2023). Nutritional and phytochemical composition and associated health benefits of oat (*Avena sativa*) grains and oat-based fermented food products. *Hindwai*, Volume 2023, Article ID 2730175, 16 pages.
48. Khan, S., Qurainy, F.A., Anwar, F. (2009). Sodium azide: - a chemical mutagen for enhancement of agronomic traits of crop plants. *Environ. We Int. J. Sci. Tech*. 4 ,1- 21 (its relevance to the society).
49. Mahato, U., & Kumar, K. (2024). Cytological study of medicinally important plant Fennl (*Foeniculum vulgare Mill*.) *The Biobrio*: An International Quarterly Journal of Life Sciences, ISSN: 2393-9508e-ISSN: 2582-4902 11 (1&2): 1165-1167.
50. Firdaus, S., & Kumar, K. (2021). Comparative karyomorphology studies of two species of *Crinum. Int. J. of Adv. Res*. 787-792] (ISSN 2320-5407).